A novel biomarker panel for triage of HPV positive women with high specificity for detection of clinically relevant cervical disease.

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Introduction

HR-HPV based primary cervical screening is now being implemented in several countries. Robust objective triage strategies for management of HR-HPV positive women are needed.

The objective of this study was to identify proteins involved in innate immunity at epithelial surfaces which could be used as biomarkers to risk stratify HR-HPV positive women.

Methods

Samples- Liquid based cytology (LBC) samples were obtained from both screening and colposcopy populations from the Scottish HPV archive.

HPV typing was done using the Abbott rtHPV test.

Protein extraction was done using chloroform isopropanol method (Canham *et al*, 2014).

Chemokine levels were measured using ProcartaPlex kit (Affymetrix eBioscience, UK) according to manufacturer's guidelines.

Results

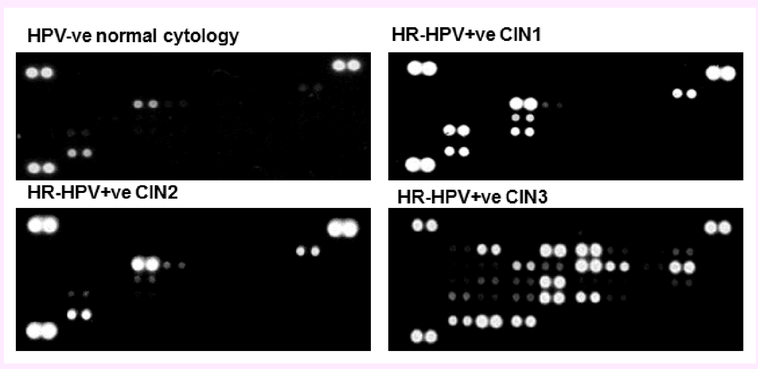
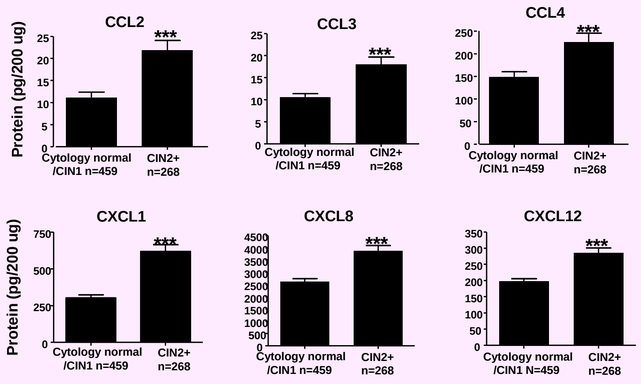


Figure 1. Differences in chemokine proteins between CIN3 and all other groups. Pooled protein extracts from LBC samples (10mg of protein from each of 9 women) from women with different grades of disease and normal controls were analysed using chemokine proteomic array (R&D Systems, UK). **Left-** Each chemokine is printed in duplicate in the array. Patients with CIN3 showed a very different pattern of chemokine expression from any other group. **Right–** Expression level of chemokines in HR-HPV positive CIN3 relative to +ve control dots.



	Sens	Spec	PPV	NPV
CXCL1	55.5%	75.6%	68.8%	63.7%
CCL2 and CXCL1	65.5%	72.4%	69.6%	68.5%
CXCL1 and CXCL8	62.2%	76.4%	71.8%	67.6%
CCL2, CXCL1 and CXCL8	71.4%	65.0%	66.4%	70.2%
CCL2, CCL3, CCL4, CXCL1, CXCL8, CXCL12	67.2%	72.4%	70.2%	69.5%

Figure 2. Six chemokines are upregulated in HR-HPV positive women with CIN2+. Results of a case control study of LBC samples from HR-HPV positive women with CIN2+ (cases, n-=268) and cytology normal or CIN1 (controls, n=459) using ProcartaPlex kit. **Left-** Barcharts indicating levels of protein, *** = $p<0.001$ (Kruskal-Wallis test with Dunn's multiple comparison post-test). **Right–** Multivariate analysis of different predictive models to assess sensitivity (Sens), Specificity (Spec), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for detection of CIN2+.

Outcome

A panel of chemokines has been identified which could serve as an objective biomarker for HPV infection associated with CIN2+. This has potential as a triage strategy for HPV primary screening.

References: Canham M, Charsou C, Stewart J, Moncur S, Hoodless L, Bhatia R, Cong D, Cubie H, Busby-Earle C, Williams A, McLoughlin V, Campbell JD, Cuschieri K, Howie S. Increased cycling cell numbers and stem cell associated proteins as potential biomarkers for high grade human papillomavirus+ve pre-neoplastic cervical disease. PLoS One. 2014 Dec 22;9(12):e115379. doi: 10.1371/journal.pone.0115379.